critical for memory consolidation. Neuronal replay may perhaps allow for a dialogue between the hippocampus and neocortex during sleep. Yet other mechanisms, particularly within the neocortex, might also make a critical contribution to consolidation. For example, during sleep and specifically during SWS, the number of cortical synapses may be decreased [14]. Synaptic downscaling could erode certain memories, making them more easily forgotten, while, other memories are relatively strengthened [15]. During sleep there are changes in the functional organization of the neocortex that are dependent upon neuronal activity. Blocking neuronal activity within the visual cortex prevents the sleep-dependent organization of functional cortical columns [16]. Thus, several different biological mechanisms may be critical for the processing of memories over a night of sleep. An important challenge for future work is to understand the contribution of these mechanisms, and how they interact.

Changes in neuronal replay may alter the fate of a memory. For example, a reward experienced during learning can enhance subsequent neuronal replay and memory retention [17]. The effect of external cues, either olfactory or auditory, may provide a simple, robust experimental model to understand how neuronal replay can be controlled, and the destiny of a memory altered. At times, neuronal replay may be enhanced by the engagement or activation of additional neuronal processes, such as those associated with reward. Equally, at other times, neuronal replay may be subjected to an inhibitory control with the activation of neuronal processes preventing replay. So, for example, disruption of the prefrontal cortex can allow the consolidation of some memories ([18,19]; for a review see [2]). Appreciating that neuronal replay is critical for memory consolidation opens up the possibility of understanding how memory consolidation is controlled, and how the fate of a memory is determined.

In summary, the recent study by Fuentemilla *et al.* [5] demonstrates that hippocampal reactivation is critical to memory consolidation, and serves to highlight the functional relevance of neuronal reactivation to human memory processing. Understanding how neuronal reactivation is controlled and how it relates and interacts with other mechanisms of memory consolidation are important challenges that we face as we attempt to understand the biology of memory.

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Membrane Traffic: The Exocyst Meets the Cell Cycle

A new study describes a novel regulatory event that results in the inhibition of exocytic transport of a specific class of Golgi-derived vesicles during mitosis. The mechanism of inhibition is shown to involve direct phosphorylation of a subunit of the exocyst by a specific cyclin-dependent kinase complex.

Patrick Brennwald

The ability of eukaryotic cells to expand and remodel their surfaces in precise and well-orchestrated ways is central to their ability to grow, divide, and organize themselves in ways that allowed for the development of multicellular animals [1]. Appropriately timed and spatially restricted delivery of new membrane components by exocytic fusion of vesicles with the plasma membrane underlies morphogenic events, such as rapid surface growth and cytokinesis. However, in actively growing and dividing cells, different stages of the cell cycle come with distinct needs in terms of surface delivery and therefore these two events must be carefully coordinated. Recent work from the Guo lab [2] now provides new insights

into both a novel point of regulation as well as a molecular mechanism by which this regulation appears to be carried out. These results point to a multiprotein tethering complex, known as the exocyst, as a key target for cell-cycle regulation and add to a growing list of signaling pathways that converge on this fascinating complex.

To explore the possibility that membrane transport to the cell surface might be differentially regulated during specific stages of the cell cycle, Luo *et al.* [2] made use of the budding yeast, *Saccharomyces cerevisiae*, in which both the cell cycle and exocytic processes have been extensively characterized [3,4]. Moreover, both of these processes are highly conserved amongst budding yeast and other eukaryotes; therefore, the yeast model represents an extremely important tool for understanding how these events are carried out in animal cells.

To determine whether exocytic transport might be regulated during the cell cycle, Luo *et al.* [2] utilized a series of yeast strains with mutations known to cause cells to arrest at distinct stages of the cell cycle and characterized them for defects in the release of either of two proteins known to depend on exocytosis for their secretion from the cell. This turned out to be key to the success of the study: while secretion of one of the cargos — the sucrose-hydrolyzing enzyme invertase — was found to be normal in all the cell cycle mutants examined, the other cargo — the cell wall protein Bgl2 — was found to specifically accumulate in a *cdc20-1* strain containing a mutant form of the yeast homolog of maturation promotion factor (MPF). This mutant is known to cause a block in the progression from metaphase to anaphase during mitosis. Electron microscopy of the *cdc20-1* mutant demonstrated a pronounced accumulation of 80–100 nm vesicles that are normally associated with transport from the Golgi to the cell surface. Since invertase and Bgl2 are known to be carried by distinct types of vesicles, these findings suggested the existence of a cell-cycle-specific signaling mechanism that affects docking and fusion of a particular class of post-Golgi vesicles.

The authors go on to determine the molecular basis of this regulation by identifying a cell-cycle-specific phosphorylation event mediated by cyclin-dependent kinase 1 (Cdk1) that is likely responsible for this phenomenon. The Exo84 component of the multisubunit exocyst complex appeared to be the relevant Cdk1 target as its sequence contained a number of predicted Cdk1 consensus phosphorylation sites. In fact, analysis of synchronized cells demonstrated that Exo84 was specifically phosphorylated *in vivo* during M phase when the B-type cyclin Clb2 is expressed. The relevance of Clb2–Cdk1-mediated phosphorylation and the consensus Cdk1 sites was examined *in vitro*: the purified Clb2–Cdk1 complex (but not the Clb5–Cdk1 complex) phosphorylated recombinant Exo84, but not a mutant form of the protein, Exo84-A, lacking the five Cdk1 consensus sites. The connection of Exo84 phosphorylation to the vesicle accumulation and Bgl2 secretion defect was made by examining the effect of the *exo84-A* allele on the cell cycle arrest in the *cdc20-1* mutant. Strikingly, both the vesicle accumulation and secretion defect are completely prevented in response to loss of the Cdk1 consensus sites in Exo84. Surprisingly, only a very modest effect on daughter cell growth is observed in cells expressing the *exo84-A* mutant in the absence of endogenous Exo84, suggesting that other mechanisms for coordinating bud growth with the cell cycle are likely present.

What effect does phosphorylation of Exo84 have on its function in exocytosis? Exo84 is a subunit of an octameric complex that is important for docking and fusion of Golgi-derived vesicles with the plasma membrane. Current models suggest that all eight subunits need to be present for full exocytic function of the complex. Therefore, any disruption of the assembly of the intact complex is likely to significantly disrupt function. To determine whether Exo84 phosphorylation had any effect on its assembly into the exocyst complex, Luo *et al.* [2] examined the effect of Exo84 phosphorylation site mutants on its incorporation into intact complexes by immunoprecipation studies. In addition to alanine substitutions of the Cdk1 consensus sites (Exo84-A), phosphomimetic glutamic acid substitutions (Exo84-E) were also examined for their effects on assembly. The results demonstrated that, while the Exo84-E mutant had clear defects compared with wild-type Exo84 in co-associating with two of the other exocyst components, the Exo84-A mutant showed a pronounced increase in association with the same two components. This supports the model that phosphorylation of Exo84 antagonizes its association with other exocyst subunits, which may underlie the mechanism by which Cdk1 inhibits trafficking at the metaphase–anaphase transition.

The Bgl2- and cell-cycle-specific defects of this pathway are reminiscent of the phenotypes reported in two other studies. One study involves the cell polarity determinant Cdc42, a Rho family GTPase that is an important regulator of exocyst function during bud emergence. The *cdc42-6* mutant strain has a Bgl2 secretion defect (but no invertase secretion defect) that is observed only early in mitosis during bud emergence [5]. The second study [6] involves the Exo70 exocyst subunit, which is a direct effector of Cdc42 function in exocytosis [7]. He *et al.* [6] demonstrate that phenotypes of the *exo70-38* allele have both cell-cycle- and cargo-specific secretory defects that are virtually identical to those of *cdc42-6*. Therefore, like the Cdk1–Exo84 pathway, the Cdc42–Exo70 pathway appears to signal to a specific class of vesicles at a particular point in the cell cycle. The mechanism behind this discrimination is presently unknown and is especially intriguing since the release of both Bgl2 and invertase has been shown to otherwise involve the same general exocytic machinery (Figure 1).

In addition to the Cdk1 regulation, two other recent reports described regulation of the exocyst complex by phosphorylation. Saltiel and colleagues [8] showed that in vertebrates the interaction of the Sec5 subunit of the exocyst with the small GTPase RalA is negatively regulated by protein kinase C (PKC) phosphorylation. In contrast, positive regulation of exocyst function by phosphorylation has also recently been demonstrated. In this case, phosphorylation of the Exo70 subunit of the exocyst by the MAP kinase ERK leads to an increase in exocyst assembly, invadopodia formation, and matrix metalloprotease secretion [9].

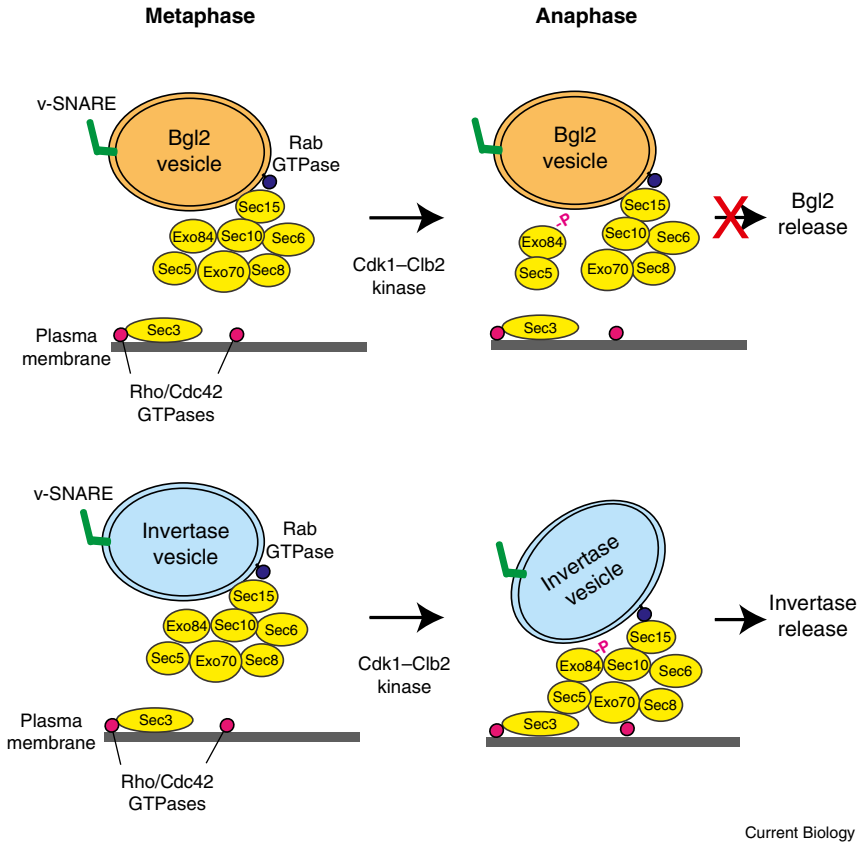

Figure 1. Differential effect of Cdk1–Clb2-mediated phosphorylation of Exo84 on two classes of post-Golgi vesicles.

During most of the cell cycle, vesicles carrying Bgl2 and invertase from the Golgi to the cell surface utilize a similar machinery comprising v-SNAREs (Snc1/2 in green), Rab GTPases (Sec4 in purple), Rho/Cdc42 GTPases (in red), and the eight-subunit Exocyst complex (yellow). During the metaphase–anaphase transition, Cdk1–Clb2 phosphorylates the Exo84 subunit of the exocyst. This leads to a destabilization of the interaction of Exo84 with the Sec10 component of the exocyst complex and a specific inhibition of Bgl2 release, while invertase release is unaffected. The effect of Exo84 phosphorylation on the assembly state of the exocyst subunits that do not interact with Exo84 remains to be determined. Both pathways depend on the same exocyst, Rab GTPase, and v-SNARE proteins, although differences in the amounts or activation states of these proteins could account for the different sensitivity of the two classes of vesicles to Exo84 phosphorylation.

Given the numerous ways that its function is regulated — i.e. by Rab, Ral and Rho GTPases, Cdk, PKC and MAP kinases — it is clear that the exocyst complex represents an important target for cells to modulate both the rate and location of cell-surface transport events. This regulation can serve to direct surface growth spatially by increasing the rate of transport at specific sites marked by Cdc42/Rho GTPases [10] or by generating specific membrane protrusions in the case of ERK [9].

Clearly regulation of specific protein–protein interactions within the exocyst complex is an important mechanism by which the specific function(s) of this complex are regulated by external signaling pathways. Nevertheless, this still begs the question of the precise functions of the exocyst during exocytic vesicle docking and fusion. The simplest model, albeit unsatisfying, is that the eight member arrangement of helical rods serves as a physical bridge between the vesicles containing Rab/Ral GTPases and the plasma membrane, thereby physically beginning to dock or tether the vesicles to their intended target membrane. However, a growing body of data supports the notion that members of the CATCHR family of tethering complexes — including the exocyst — play important roles in regulating the assembly of the SNARE complexes that act directly in the downstream fusion of the vesicle with the target membrane (reviewed in [11,12]). In this view the exocyst may therefore have an important role in catalyzing formation of fusion-competent SNARE bundles. The effects of specific regulatory inputs, such as phosphorylation or Cdc42/Rho binding, can be viewed as destabilizing or strengthening interactions within the complex that either inhibit or promote this catalytic function. Future studies will no doubt begin to unravel these molecular insights into the functions of the exocyst and CATCHR complexes and how these regulatory signals affect them.

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